COMPONENTS OF *Libanotis intermedia* RUPR. FRUITS*

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From the light petroleum extract of *Libanotis intermedia* RUPR. fruits paraffins, monoterpenic hydrocarbons, edultin, substance $C_{15}H_{20}O_3$, fats as the main components, a new sesquiterpenic hydroxy ester $C_{20}H_{32}O_3$, and a mixture of phytosterols (β-sitosterol, stigmasterol, campesterol) were isolated. In the essential oil the presence of monoterpenic hydrocarbons, β-elemene and terpinen-4-ol was established.

The components of *Libanotis* genus (*Umbellifereae*) are relatively little known[1]. We now carried out an analysis of the light petroleum extract of the fruits of *L. intermedia* RUPR., a biennial and perennial plant growing in the temperate zone. In literature only the presence of auraptene, 7-isopentenyloxycumarin, and an essential oil in the fruits is mentioned[2]. In subsequent paper[3] the isolation of three sesquiterpenic hydrocarbons of undetermined structure has been described, which most probably, were not pure.

The light petroleum extract was chromatographed on deactivated silica gel. The early fractions, eluted with light petroleum and representing 23% of the extract, contained hydrocarbons which gave a mixture of monoterpenic hydrocarbons as the main fraction on distillation. According to GLC analysis this mixture contained sabinene (34%), limonene (21%), α-pinene (14%), γ-terpinene (10%), α-felandrene (4·6%), Δ_3-carene (4·3%), *p*-cymene (3·5%), myrcene (1·6%), camphene (1·4%), and traces of β-pinene. The distillation residue contained paraffinic hydrocarbons from C_{21} to C_{31}, with prevailing C_{29} (GLC) component and a small amount of sesquiterpenic hydrocarbons two of which composed the main fraction. Owing to the small amount available, this mixture was not further investigated.

In the fractions following the hydrocarbon mixture a crystalline substance was present, which we isolated by thin-layer chromatography. A comparison of its IR and MS spectra with those of an authentic sample identified the substance as edultin[4] [8-(1-acetoxyisopropyl)-9-angelyloxy-2*H*-furo(2,3-*h*)-1-benzopyran-2-one]. The

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more polar component which accompanied edultin, also isolated by TLC, contained a carbonyl and a hydroxyl group according to its IR spectrum. On the basis of its high resolution mass spectrum the substances had the composition $C_{15}H_{20}O_3$. The major part of the extract (almost 60%) was composed of fat containing fractions. The representation of single fatty acids was determined by the conventional GLC analysis of corresponding methyl esters. The main components were $C_{18:1}$ and $C_{18:2}$ acids. In the last fractions containing fats a further substance appeared with a different stretching vibration frequence of its carbonyl group (1713 cm^{-1}). Rechromatography of the fractions with the highest content of this substance gave a crystalline compound of the composition $C_{20}H_{32}O_3$ (high resolution mass spectrum) which displayed the presence of a hydroxyl group (3625 cm^{-1}) in its IR spectrum in addition to the carbonyl group. On the basis of a preliminary ^{1}H-NMR spectrum it is most probably a sesquiterpenic diol esterified with C_5 acid. The last chromatographic fraction contained a mixture of phytosterols, *i.e.* β-sitosterol, stigmasterol and campesterol, as shown by combined GLC/MS. This mixture was accompanied by traces of 24-ethylcholestane-3-diol.

On distillation of the fruits with water about 2% of an essential oil can be obtained, containing predominantly monoterpenic compounds. The mixture of monoterpenic hydrocarbons obtained by chromatography on silica gel displayed a similar composition as hydrocarbons present in the extract. The difference in quantitative composition could be observed mainly in γ-terpinene which was present in the extract in a much higher amount than in the essential oil, and which is converted to *p*-cymene and terpinolene, most probably during the preparation of the essential oil. Among sesquiterpenic hydrocarbons β-elemene was predominant, accompanied by about 18 hydrocarbons, all in very low concentrations. Among oxygen-containing substances terpinen-4-ol was identified by GLC/MS. However, a considerable part of the oxygen-containing substances was destroyed during chromatography, even when this was carried out under very mild conditions.

EXPERIMENTAL

The infrared spectra were measured in chloroform solution on a Zeiss UR-20 (Jena) instrument and the optical activity was determined with an objective Perkin-Elmer 141 polarimeter. Other conditions were identical with those described earlier[5].

Chromatography of the Light Petroleum Extract

Ground fruits of *L. intermedia* (4·75 kg) obtained from a plant cultivated in the Garden of Medicinal Plants, Academy of Medicine, Poznań (the seeds were obtained from the All-Union Research Institute for Medicinal and Aromatic Plants (VILAR), Moscow), were extracted with light petroleum. After evaporation of the solvent 382 g of an extract were obtained, which was diluted with light petroleum (300 ml) and chromatographed on a column of deactivated silica gel (11 kg), using light petroleum with increasing concentration of ethyl acetate (1 – 8%) for elution. Individual fractions collected were of 10 l volume.

Hydrocarbons. Fractions 1—3 (light petroleum; 83 g) were distilled at 15 Torr using a 10 TP column. The distillation fraction of b.p. up to 100°C (60·4 g) was analysed by GLC on a Perkin-Elmer F 11 instrument provided with FID; column length 4 m, i.d. 2·2 mm, support Chromosorb W (80—100 mesh) wetted with 10% Carbowax 400, temperature 80°C, nitrogen as carrier gas. Identification of sabinene was carried out at 70°C. Retention times were referred to limonene and the amount of individual components was determined planimetrically. The distillation residue (10 g) was chromatographed on 80 g of silica gel impregnated with silver nitrate. Light petroleum eluted paraffins (5·5 g) and ether unsaturated compounds (2·8 g). The mixture of paraffinic hydrocarbons was analysed by GLC using a Pye 104 apparatus, provided with FID; column length 1·5 m, i.d. 6·5 mm, carrier Gas-chrom Q wetted with 3% of SE-30; 230°C, nitrogen as carrier gas. Identification was carried out by comparison with a standard mixture of n-paraffins (C_{24}—C_{30}).

Edultin. The combined fractions 4—9 (light petroleum; total weight of the residue 6·44 g) were separated by preparative chromatography on silica gel layers. In a typical experiment 20 mg of the residue were applied on a 20 × 10 cm plate (layer thickness 0·2 mm) and run in benzene–ether 8 : 2. After elution and evaporation of the solvent the less polar component, displaying intensive light fluorescence, afforded crystals of m.p. 142—146°C (benzene–light petroleum); IR and MS spectra were identical with the spectra of authentic edultin.

Substance $C_{15}H_{20}O_3$. The more polar substance, obtained from the above mentioned preparative thin-layer chromatography, had m.p. 140—141°C (light petroleum–ether). Molecular mass (high-resolution mass spectrometry): 248·1413; calcula'ed; 248·1412 for $C_{15}H_{20}O_3$. IR spectrum: 1740 cm^{-1} (CO), 3595 and 3410 cm^{-1} (OH) and an intensive broad band at 1685 cm^{-1}.

Fats. A part (100 mg) of the chromatographic fraction 10—21 (light petroleum–ethyl acetate 1—4%; 215·5 g) was converted to fatty acid methyl esters[5]. Conditions of GLC: instrument Perkin-Elmer F 11, column length 150 cm, i.d. 4 mm, carrier HMDS Chromosorb W 80/100, impregnated with 20% diethylene glycol succinate; 170°C; nitrogen as carrier gas. Using planimetry for comparison with standards methyl esters of fatty acids the percentual composition of acids was determined: $C_{16:0}$ 4·13%, $C_{16:1}$ 0·4%, $C_{18:0}$ 1·3%, $C_{18:1}$ 54·61%, $C_{18:2}$ 38·51%.

Sesquiterpenic hydroxy ester. Combined fractions 22—25 (light petroleum–ethyl acetate 4—7%, residue 13·6 g) were rechromatographed on silica gel (550 g). On elution with a light petroleum–ethyl acetate (97 : 3) mixture a substance was obtained from the medium fractions, having m.p. 46—47°C, $[\alpha]_D^{20}$ −198° (*c* 1·17, chloroform). IR spectrum: 848, 1650, 1713, 3652 cm^{-1}; UV spectrum: λ_{max} 218 nm ($\log \varepsilon = 3{\cdot}94$). Mass spectrum (high resolution): 320·2351; for $C_{20}H_{32}O_3$ calculated: 320·2551.

Phytosterols. Fraction 29 (elution with light petroleum–8% of ethyl acetate, 4·56 g) afforded on rechromatography on silica gel (400 g) a substance of m.p. 137—139°C in the front fractions. Using combined GLC/MS (Pye 104 instrument: column length 160 cm, i.d. 4 mm, support GAS-CHROM Q 80/100 mesh, impregnated with 3% SE 30, temperature 220—250°C/1° min, carrier gas nitrogen. Temperature of the ionic source 190°C, electron energy 70 eV) the presence of β-sitosterol, stigmasterol and campesterol was determined in a 5 : 3 : 1 ratio, as well as traces of 24-ethylcholestan-3-ol.

Essential Oil

Ground fruits (1 200 g) were distilled in the presence of water for 8 h. The separated, dried essential oil (23·6 g) had d_4^{20} 0·8975, n_D^{20} 1·4842 and $[\alpha]_D^{20}$ +24 (substance). The essential oil (17·2 g) was chromatographed on silica gel, deactivated by addition of 14% of water. Elution with light

petroleum gave a mixture of hydrocarbons (6·1 g) which on vacuum distillation at 20 Torr (using an all-glass column of 10 TP) afforded a fraction of monoterpenic hydrocarbons (4·1 g) and a distillation residue (1·8 g). The mixture of monoterpenic hydrocarbons was analysed in the same manner as the hydrocarbons from the extract. The distillation residue was analysed on the same instrument, using a capillary column (50 m length, impregnated with the OV-17 phase at 210°C); the main component displayed the same elution time as β-elemene. Its isolation was carried out by TLC on layers of silica gel impregnated with 10% silver nitrate, using light petroleum–ether 97 : 3 for development. The IR spectrum of the isolated substance was identical with that of a standard.

Elution with ether gave a mixture of oxygen-containing substances (4·8 g). GLC analysis: column length 2 m, i.d. 4 mm, support GAS-CHROM Q impregnated with 3% QF-1, column temperature 130°C, carrier gas nitrogen. Using combined GLC/MS (for conditions see[6]) the main component was identified as terpinen-4-ol on the basis of comparison of a spectrum with that of a reference sample.

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REFERENCES

1. Hegnauer R.: *Chemotaxonomie der Pflanzen*, Bd. 6, p. 612. Birkhäuser, Basel 1973.
2. Prokopenko A. P.: Rast. Resur. *2*, 201 (1966).
3. Solodovnichenko N. M.: Khim. Prir. Soedin. *6*, 768 (1970).
4. Mitsuhashi H., Itoh T.: Chem. Pharm. Bull. *10*, 511, 514 (1962).
5. Motl O.: This Journal *37*, 3637 (1972).
6. Motl O., Trka A.: J. Soc. Cosmet. Chem. *24*, 747 (1973).

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